

Fertility and Embryo Development of Broiler Hatching Eggs Evaluated with a Hyperspectral Imaging and Predictive Modeling System

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Abstract: A hyperspectral imaging system and a predictive modeling technique was evaluated for determining fertility and early embryo development of broiler chicken hatching eggs. Twenty-four broiler eggs were collected (12 fertile, 12 infertile) for each of 8 replicate trials ($n = 192$) and imaged on Days 0, 1, 2 and 3 of incubation for training and model validation. Three replications of 30 eggs each (fertile and infertile eggs randomly mixed) were collected and imaged as above for model verification ($n = 90$). Eggs were backlit and positioned below and vertical to the imaging system (lens, spectrograph and CCD camera). Spatial and spectral data from approximately 400-1000nm were collected for each egg on each day of incubation with refinement to 550-899nm. A Mahalanobis Distance (MD) supervised classifier was trained with spectral data from the first 5 replicate sets of eggs, then Principal Component Analysis (PCA) was performed. This model was applied to the next 3 sets for model validation and then to the three 30 egg sets for verification. Fertility was confirmed on Day 5 of incubation by candling and breakout. The MD/PCA model predictions for the 3 validation sets of eggs were: 71% accuracy for Day 0; 63% for Day 1, 65% for Day 2 and 83% for Day 3. For the 3 sets of verification eggs, the MD/PCA model accurately predicted 46/90 on Day 1 and 45/90 on Day 3. The data indicate that the particular MD/PCA model used is not appropriate for predicting fertility and early development.

Key words: Broiler hatching eggs, fertility, hyperspectral imaging, predictive modeling

Introduction

The US produced more than 12 billion poultry hatching eggs in 2007 (USDA, 2008a). For broilers, the 19 major producing states in the US in 2006 set just over 11 billion eggs and hatched just over 9 billion chicks, an 82.2 % hatch rate (USDA, 2005). Approximately 92.5% of fertile eggs set hatch, so 7.5% of unhatched eggs are due to early or late embryo mortality (Zakaria, *et al.*, 2005). Therefore approximately 10% of eggs set are infertile, which is more than a billion eggs in the US annually.

The number of hatcheries has decreased while the capacity of remaining hatcheries and the capacity of individual hatchers within the hatchery has increased toward full capacity (USDA, 2008b). More crowded hatchers may decrease hatchability by up to 2% due to decreased ventilation and higher temperatures in the egg racks, (French, 1997). Actual incubator temperatures may be even higher than the incubator thermometer measurement, as Mauldin and Buhr (1995) reported thermometer readings of eggs were 0.2-0.4°C higher than the incubator thermometer. Unhatchable eggs, whether infertile eggs set or embryos dying in the incubator, make up more than 10% of eggs in a hatcher. These eggs are likely to harbor and grow pathogenic bacteria or molds, contributing to cross-contamination when these eggs build up pressure during interior decomposition and erupt as

“exploders” in the incubator. Although a few of these eggs may be removed during candling of small lots to determine flock fertility, the vast majority remain until day 18 of incubation.

Potentially, detection and removal of the infertile eggs prior to incubation could result in cost efficiencies for hatcheries, better chick quality and lower pathogen contamination of chicks. Although impractical due to increased labor costs and decreased hatchability, the removal of infertile or early dead or non-viable embryos during the first 3 days of incubation could provide some benefit to specialized hatcheries, such as those that export pre-incubated eggs.

Previous researchers have been interested in nondestructive devices and methods to evaluate internal egg quality for many years. Most of this research was to detect blood spots or other internal defects in table eggs (Brant *et al.*, 1953; Patel *et al.*, 1996; Patel *et al.*, 1998; Schouenberg, 2003). A number of these methods were reviewed by De Ketelaere *et al.* (2004). For hatching eggs, prior reports show that efforts have been made to detect egg fertility prior to or during early incubation. Non-commercial research methods of monitoring embryo development include ultrasonograph, magnetic resonance imaging, acoustic resonance frequency and ultrasound (Klein *et al.*, 2002; Coucke *et al.*, 1997; Schellpfeffer *et al.*, 2005). Kulsea published a review of embryo imaging research methods in 2004.

Methods of fertility and embryo development that could potentially be used commercially typically involve light transmission and spectral analysis, usually as part of a machine vision system (imaging) and computer analysis. Das and Evans (1992a), using machine vision and histogram analysis, detected fertile embryo development at 3 days of incubation with 88-90% accuracy; day 4 incubation accuracy was 96-100%. Using the same vision system but a neural network classification system to detect embryo development, they reported over 93% accuracy on days 3 and 4, but just over 67% at day 2 of incubation (Das and Evans (1992b). An imaging system was used to detect embryo development at 4.5-5 days of incubation using two light wavelengths (Bamelis *et al.*, 2002). Liu *et al.* (2004). An imaging system was developed and patented predominately for late incubated eggs at day 18, during transfer to the hatching cabinet (Chalker and Hutchins, 2003). A preliminary experiment found that hyperspectral imaging could detect embryo development at 3 days of incubation (Smith *et al.*, 2005).

Potentially, the detection and removal of infertile eggs prior to incubation could benefit the poultry industry by:

- 1) Allowing hatcheries to operate more efficiently by only setting hatchable eggs;
- 2) Allowing hatcheries to set less eggs per setter to increase hatchability; or
- 3) Preventing or minimizing exploder eggs in the incubators, decreasing egg and chick pathogen or mold contamination.

Detecting infertile or non-viable embryos during the first 3 days of incubation would be of less value to the industry but could still benefit specialized hatcheries. Therefore, the objective of this experiment was to evaluate the feasibility of hyperspectral imaging and predictive modeling for 1) determining hatching egg fertility prior to incubation and 2) examining embryo development during the first 72h of incubation.

Materials and Methods

Broiler chicken breeder hens (Ross 708 strain) were separated into two groups at 26 weeks of age. One group was placed into floor pens with roosters (1:10 rooster:hen ratio) to provide fertile eggs. Hens from the other group were placed in individual cages without roosters or artificial insemination to provide infertile (control) eggs. Both groups were fed a restricted diet of a typical broiler breeder ration. Light was maintained at 14h per day. Eggs were collected for this experiment from weeks 29-36 as measured by age of flock.

Eggs were collected twice daily, with the second collection timed to ensure eggs were less than 4h post-lay at time of initial imaging. On Day 0 (after initial imaging) eggs were immediately placed in a small commercial incubator set at 38°C with 55% relative humidity, and turned 3 times per hour. Eggs were

removed on Days 1, 2 and 3 of incubation for imaging. The imaging process was approximately 30 min., then eggs were immediately replaced in the incubator. After 5 days of incubation, eggs were candled and broken out to determine fertility or embryo viability.

Fertile and infertile eggs, 12 of each, were collected on each of 8 days ($n = 192$). The first 5 groups were used as training data ($n = 120$) and the last 3 groups were used for model validation ($n = 72$). Three replicate groups of 30 eggs each, with fertile and infertile eggs randomly mixed, were also collected, incubated and imaged at the times described above for a blind verification of the predictive model ($n = 90$).

Eggs were imaged four at a time using transmitted illumination provided by a single 150W tungsten-halogen lamp. Cross contamination of light was prevented by using a 5-cm tall divider in an X-shape. A hyperspectral imaging system, capable of collecting imagery from 400-1000 nm, was positioned directly over top of the eggs at a 28-cm working distance. The imaging system consisted of a 12-bit digital camera (SensiCam QE, Cooke Corporation, Romulus, MI) with a 1376 x 1040 Peltier-cooled CCD. The camera's fore-optics consisted of a prism-grating-prism (ImSpector V10E, Specim, Oulu, Finland), focal plane array (Institute for Technology Development, Stennis Space Center, MS) and 17-mm front lens (Xenoplan 1.4/17mm C-Mount, Schneider Optics, Hauppauge, NY). System integration time was set at 200 ms and the front lens' f-stop set at 2.8. Camera binning was set at 4 x 2 and 250 lines were collected per image, resulting in an output image of 344 columns x 250 rows x 520 spectral bands.

Images were pre-processed by calibrating to percent transmission. Spectral subsets (550-899nm) of the first 5 sets were sorted into mosaics based on their time of incubation (Day 0-3) and served as training data for subsequent classification. Likewise, mosaics were generated for the 3 validation sets, as well as the 3 blind verification sets. Principal Component Analyses (PCA) were performed on the training data from the egg feature space only and spectral signatures collected for application to the validation and blind sets. Signatures for infertile and fertile eggs were derived by collecting the spectral means from the centermost portions of each egg. This process was repeated for each mosaic (Day 0-3) from batches one through five, thus four sets of infertile and fertile signatures were generated. These time-specific and fertility-specific signatures were used to train a Mahalanobis Distance (MD) supervised classifier, which was applied to each time-specific calibrated mosaic. Images were post-classified by applying a majority analysis to each classified egg, resulting in a single infertile/fertile class assignment for each egg.

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Table 1: Mahalanobis Distance classifier predictive model and Principle Component Analysis results of 90 broiler hatching eggs imaged by a hyperspectral system (n=90 eggs and 360 images)

	Initial (Day 0)		Candle (Day 5)		Day	Eggs	Imaging and Analysis			
	Trial	Fertile	Infertile	Fertile			Infertile	Accurate ¹	False+ ²	False- ³
		(No.)		(No.)						
Subtotal	1	16	14	14	16	0	30	21	4	5
						1	"	15	11	4
						2	"	13	11	6
						3	"	15	8	7
							120	64	34	22
Subtotal	2	17	13	17	13	0	"	15	6	9
						1	"	18	9	3
						2	"	16	5	9
						3	"	15	7	8
							120	64	27	29
Subtotal	3	13	17	12	18	0	"	11	11	8
						1	"	13	13	4
						2	"	15	10	5
						3	"	16	9	5
							120	55	43	22
Totals	All	46	44	43	47		360	183	104	73
Percent								50.8	28.9	20.3

¹System detected egg as fertile or infertile correctly as identified by 5 day candle and breakout

²System detected an infertile egg as fertile, ³System detected a fertile egg as infertile

Results and Discussion

The birds used in this study displayed a high level of egg fertility, probably due to the relatively young age of the hens and the optimum conditions of a research farm environment. From the 90 eggs used as a model validation, 46 were from the fertile hens and 3 eggs were candled as infertile, yielding a 93.5% flock fertility rate (Table 1).

The PCA/MD model applied to the training data (first 5 batches of known eggs, 120 total) accurately predicted fertility/development at 79% on Day 0, 81% on Day 1, 82% on Day 2 and 93% on Day 3 (data not shown). The model was applied for validating the subsequent following 3 sets of known eggs (72 in total) that were collected and incubated. The model produced a lower level of accuracy with results on Day 0 at 71%, Day 1 at 63%, Day 2 at 65% and Day 3 at 83% (data not shown). The model as developed was then applied to the verification sets (3 groups of 30 eggs each, random mix of fertile and infertile eggs, unknown to the system beforehand) produced an overall accurate prediction of 50.8% (shown in Table 1).

Although the results from the training data were relatively low (79-93% accuracy) it seemed further study was warranted. The cause of the continued decrease in accuracy results for both the validation set (63-83% accurate) and then the verification sets (50.8% accurate overall) was not determined. Results obtained in this experiment in regard to accuracy of detecting embryo development were much lower than those reported by Das and Evans (1992a, b). The mechanical imaging systems were different, but also white shell eggs were used in the previous experiments versus the brown shell

eggs used in the current study. Smith *et al.* (2005) also reported higher accuracy in detecting early embryo development than the current study. They reported testing both white and brown shell eggs, with better detection accuracy in white shell eggs. Due to the relatively small sample size and few infertile (control) eggs sampled their results must be considered as preliminary. Shafey *et al.* (2004) studied the effect of brown shell pigments on light transmission, finding that the pigments significantly affected and lowered transmission of light between 300 and 800nm. Therefore the brown shell pigments do affect the hyperspectral system as it measures light transmission between approximately 400-900nm.

Based on preliminary results in the current study and in previous research, the hyperspectral imaging system itself seems appropriate for collecting images of eggs and embryos. However, the results show that the PCA/MD model as configured for this experiment is not appropriate for determining fertility and early development of broiler hatching eggs. Further testing using other techniques to develop more accurate prediction models will be necessary for detecting early development or fertility prior to incubation.

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